SYNTHESIS AND SCREENING OF PLANT ORIGINATED NANOPARTICLES FOR RAPID PESTICIDE DETECTION ON SPINACH *(SPINACIA OLERACEA* L.*)*

RESEARCH PLAN

FOR REGISTRATION IN DOCTOR OF PHILOSOPHY

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CERTIFICATE

This is to certify that this Research Proposal entitled "**SYNTHESIS AND SCREENING OF PLANT ORIGINATED NANOPARTICLES FOR RAPID PESTICIDE DETECTION ON SPINACH (*SPINACIA OLERACEA* L.)**" by **Bhupeshkumar Nanhe,** submitted in fulfilment of the requirement for registration in Ph.D. program in **Department of Botany** under School of Sciences of JECRC University, Jaipur (RJ), during the academic year 2024, is a bonafide record of work carried out under our guidance and supervision.

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INTRODUCTION

The study of creating, manufacturing, and utilizing structures, apparatuses, and systems through the manipulation of atoms and molecules at the nanoscale—that is, with one or more dimensions of the order of 100 nanometres (100 millionth of a millimetre) or less—is known as nanotechnology (Vadia et. al., 2022). Nanotechnology, sometimes known as the science of the small, aims to comprehend atoms and molecules, the tiniest building blocks of the cosmos. Imagine being able to manipulate matter at a scale so small that a single human hair could contain millions of nanotechnology devices. Thanks to this wonderful discipline, engineers and scientists can now design and construct objects and materials with extraordinary properties that were previously only seen in science fiction (Bora et al., 2024)

A new area called forensic nanotechnology uses materials and methods at the nanoscale to improve forensic science procedures. Forensic scientists can enhance the identification and analysis of evidence in criminal investigations by utilizing the special qualities of nanomaterials and nanoparticles (Tambo, et al. 2020). Numerous applications of nanotechnology exist in the field of forensic science. These include the detection of drugs and poisoning from exhibits in the field of forensic toxicology; the creation of biosensors in the field of forensic biology to detect bodily fluid traces from forensic samples; the identification of unknown material in the field of forensic chemistry; and numerous other applications (Tambo et al., 2020).

. The aim of this research is to identify pesticides in leafy vegetables that are members of the *Chenopodiaceae* family using nanotechnology (Spinach). The most commonly consumed food is spinach, which may be found in several forms and combined in numerous ways with other dishes. In addition to having high levels of vitamins and minerals, spinach also has high levels of flavonoids, phytochemicals, and bioactive substances. The bioactive chemicals found in spinach have anti-cancer, anti-obesity, hypoglycaemia, anti-cancer, anti-obesity, and cardiovascular disease properties. Despite the many health benefits of spinach, very little of it is consumed globally. It is believed that spinach originated in Persia (Iran). The Chinese were the first to recognise it in 600 A.D., calling it Persia's herb. It was brought to northern Europe around 1100 A.D. by the Moors in Spain, from where it was initially grown by the Arabs in North Africa. It was originally noted in Germany in 1280, and by 1500, England and France were using it as a common garden produce (Lasya, 2022).

Scientific classification (Lasya, 2022)
• **Plantae Subkingdom:** Tracheobionta
• **Super division:** Spermatophyta
• **Division:** Magnoliophyta
• **Class:** Magnoliopsida
• **Subclass:** Caryophyllidae
• **Order:** Caryophyllales
• **Family:** Chenopodiaceae
• **Genus:** *Spinacia* L.
• **Species:** *Spinacia oleracea* L.

According to the APG (Angiosperm Phylogeny Group) III system, the *Chenopodioideae* subfamily of the *Amaranthaceae* family of flowering plants *Chenopodioideae* are small trees, shrubs, subshrubs, and herbs that can be annual or perennial. Typically, the leaves are flat and alternating. Frequently, the flowers are unisexual. Many species exhibit mixed inflorescences of unisexual and bisexual blooms, or they are monoecious (Ramaiyan et al., 2020). This family of vegetables is yearly grown in Jaipur, Rajasthan, India and is frequently used in cuisine by locals. In order to prevent bug and pest damage to the developing plants, leafy vegetable cultivators employ a lot of insecticides and pesticides in the various regions of Jaipur where they cultivate this vegetable. However, this poses a greater risk to those who grow the vegetables than it does to those who eat them. According to (Singh et al., 2020), the majority of people suffers with chronic diseases as a result, and some symptoms include eye irritation, headaches, dizziness, vomiting, back pain, skin irritation, eye flu, and exhaustion over extended periods of time. Fatal dose and fatal period which includes Malathion about 60 gm, Parathion 175, Diazinon 1 gm which causes harmful effect in 24 hrs (Bardale, 2011).

One of the main issues with Indian agriculture is the widespread use of pesticides, which are applied to all kinds of crops by nearly all farmers in an attempt to improve yields. However, there will always a risk to consumers—human or animal. Because pesticides are extremely hazardous by nature, their fatal amount can determine whether they induce disease, death, or other complications.

The substances used to eradicate pests are known as pesticides. Insects, rats, fungi, and other things can be considered pests. A brief summary is given to the global sales of pesticides and the research on their discovery. Over the previous ten years, at least 105 chemical pesticides have been introduced or are currently being developed. Forty-three insecticides/acaricides, twenty-one herbicides, six nematicides, forty-three fungicides, and one herbicide safener. The majority are safe for people to use and kind to the environment. Quinine outside inhibitors, or QoI, quinone inside inhibitors (QiI), succinate dehydrogenase inhibitors (SDHI), and demethylation inhibitors (DMI) are the most advanced fungicides. Fungicides with established modes of action have become less effective, leading to the introduction of new fungicides or their ongoing development that have different innovative modes of action. As opposed to organophosphorus, carbamate, and synthetic pyrethroids, nicotinic and diamide insecticides are the new trend in insecticide development (Umetsu and Shirai, 2020). The following categories apply to pesticides: **Insecticides:** these are substances used to either kill or repel insects and their relatives. Organochlorines, Carbamate, Pyrethroids, and Orga-phosphates are a few examples. **Rodenticides:** they are substances that are used to eradicate rodents, including mice, rats, and moles. Examples include Warfarin, Barium Carbonate, Strychnine, and Zinc Phosphide. **Herbicide:** these are the substances that are used to eradicate weed. Acrolein, Glyphosate, Paraquat, and other substances are examples. **Fungicides** are substances that are used to destroy mould and fungus. Examples are Sodium Azide and Thiocarbamate. Other substances include Nicotine, Lead, Copper, Mercury, and so forth (Bardale, 2011).

Although the quantity of pesticide on leafy vegetables is relatively minute, continuous ingestion of the vegetable will be enough to produce chronic poisoning and symptoms. Plant-based nanoparticles will be synthesized using green waste material based on information available. To get the tailor-made specifications of nanoparticles, characterization of the particles will be an essential stage in this endeavour. Examine how plant-based nanoparticles detect pesticides from leafy crops and work to develop a quick and simple approach for detecting pesticides in veggies. Plant based nanoparticles having more advantages as compare to nanoparticles which is synthesised from other organic and inorganic substances. Plant based nanoparticles is environment friendly which does not show any harmful effect to environment before and after its use and it is cost effective. In very minimal cost, plant based green nanoparticles can be synthesised in laboratory (Sharma and Rao, 2012).

Unlike other conventional pesticide detection methods (such as chromatography and spectroscopy), which can only be carried out in a well-equipped laboratory with high cost which

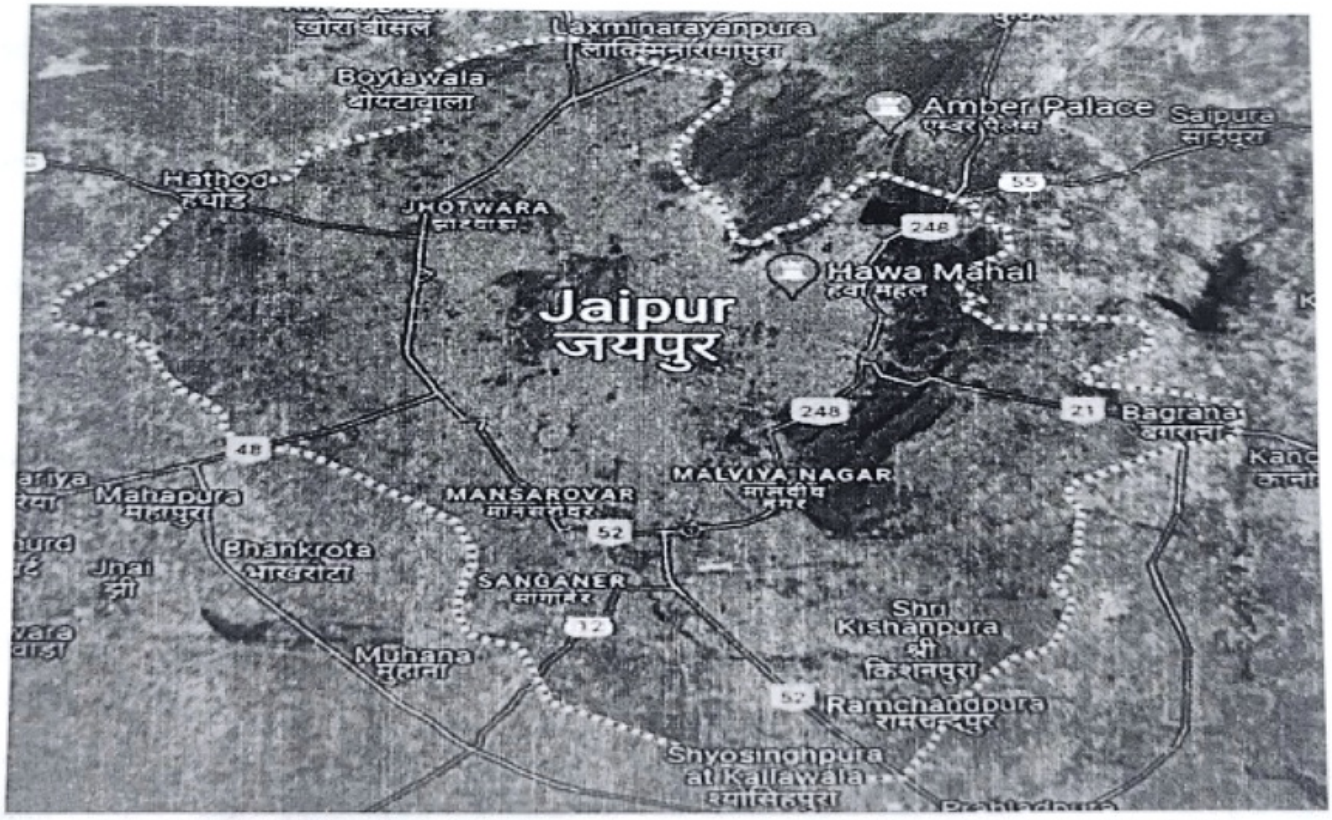
demand lot of time for detection, this approach will be helpful for detecting pesticide from vegetables at any location. It will also be easy to use and more affordable. This approach will be regarded as an on-site method for detecting pesticides from any kind of sample, and it is currently unavailable in the field of forensic science and this is the major gap of this research work. This technique will be used as part of preventative forensics, which encourages people to eat more organic vegetables.

Site Description

The region of Northeastern Rajasthan, which includes the energetic city of Jaipur, is characterised by a variety of geographical elements. India's largest state by land size, Rajasthan is renowned for its varied landscapes, which range from expansive deserts to verdant plains, as well as its rich cultural legacy and historical sites. The ancient Aravalli Mountain range, which dominates the state's terrain and climate, and the Thar Desert, one of the biggest deserts in the world, are both located there. Rajasthan is an essential region to research in order to comprehend how geography, history, and society interact in India because of its unique combination of natural and cultural features.

The 467 square kilometre city of Jaipur is bordered to the east and south by lush alluvial plains, and to the north and west by hill ranges and desert regions. Oriented from south to southeast, Jaipur has a high top. The city lies between the Nahargarh hills to the north and the Jhalana area, which is a part of the Aravalli range, to the east. (Source: https://www.tourism.rajasthan.gov.in/jaipur.html)

(Image Source https://www.google.com/search?q=spinach%20growing%20site%20in%20jaipur%20rajasthan&oldm=2&x3=true&vhid=KsJct1fwB8mTM&vssid=mosaic)



Map location of Jaipur, Rajasthan:(Source :-https://www.google.com/maps/place/Jaipur,+Rajasthan)

In this research, spinach sample from Jaipur region will be collected and pesticide will be identified from the collected spinach sample by using nanoparticles. Spinach has been selected from Jaipur areas this is because of its availability throughout the year and it is highly consumable by the peoples in their food. In Rajasthan, availability of water is minimum as compare to other states of India who grow vegetables significantly and due to this reason farmer uses extra manure and pesticide to get better yield but it might be harmful for human and animal health. The proposal is planned to research & develop instant pesticides detecting plant based nanostrips for onsite detection of spinach vegetable.

REVIEW OF LITERATURE

Abdali et al., (2024) deals with a colorimetric sensor array based on Gold (Au) nanoparticles of variable sizes to detect and discriminate pesticide residues. Gold (Au) nanoparticles with diameters of 30, 10, and 5 nm were synthesised which shows its confirmation by the results of TEM pictures, dynamic light scattering (DLS) tests, and UV–Visible spectroscopy. Different sized Gold (Au) nanoparticles were employed as sensor units to simultaneously detect and distinguish between pesticides such Bifenazate, Diazinon, Paraquat, Carbendazim, and Chlorpyrifos. The results demonstrated that, in the presence of pesticides at concentrations ranging from 25 to 800 ng/ml, different plasmonic patterns may be generated by the non-enzymatic sensor units of different sizes that were created.

Arsawiset et al., (2023) developed a paper-based analytical instrument using copper oxide nanoparticles (CuO NPs) and nanozymes for the quick identification of organophosphate pesticides in fruits and vegetables. Silica oxide nanoparticles were added to the paper-based analytical apparatus to improve the sensitivity of the experiment.

A recent advancement in green analytical techniques based on carbon dot (nanoparticles) offered new possibilities in fluorescence tests of insecticides, medications, and biomolecules. The synthesis of nanoparticles was done using both top down and bottom-up methods. They also described the pyrolysis, microwave-assisted, and hydrothermal methods of NP synthesis and the detection techniques, such as inner filter effect, Forster Resonance Energy Transfer, and photoinduced electron transfer method.

(Yusuf et al., 2022) They describe detection of pesticides, biomolecules and many other applications.

Choudhary et al., (2022) explained the use of pesticide on vegetable by agriculturist in the area of Jaipur, Rajasthan. They also mention the types of pesticide used by vegetable grower. Some common examples include Monochrotophos, Parathion, Aldrin, etc

The application of nanotechnology and nanoparticles in forensic research was explained by Tambo and Ablateye, (2020). Among many fields covered by forensic biology are single-crystalline semiconductor CdS nano slabs for explosives detection, Na-nano powder for forensic physics visualisation of latent prints, and gold nanoparticle protein nanopores for the detection of single-stranded DNA.

Liu et al., (2020) provided an overview of the many types of carbon-based nanoparticles, their synthesis process, and their applications across a range of fields. They describe in detail the synthetic processes and optical characteristics of CDs, including high absorption, photoluminescence, and phosphorescence, and gave a comprehensive overview of their classification based on the analysis of their micro-nanostructure, formation technique, and property features. Furthermore, a comprehensive summary of the most significant recent advancements in a range of domains was mentioned like optical (sensor, anticounterfeiting), energy (light emitting diodes, catalysis, photovoltaics, supercapacitors), and the exciting subject of biomedicine.

The influence of pesticide uses on farmers' health in Sri Ganganagar and Jaipur District, Rajasthan, is discussed by Singh et al., (2020). The primary and secondary data were used in the study. Using a pre-tested schedule and a personal interview with pre-planned questions, primary data were gathered from sample houses, and secondary data were gathered from Rajasthan's statistics framework. Authors considered cauliflower, cabbage, tomato and pea plant for study the practice of pesticide usage in this region and found that farmer used 100% insecticide on all plants, near about 3-20% fungicide on all four plants, 1-4% bio pest and 0.1-1.5% botanical pest used on all four plants in both Jaipur and Shri Ganganagar area and found that people suffered from chronic disease and having symptoms like eye irritation, headache, dizziness, vomit, back pain, skin irritation, eye flue, fatigue for very long period.

Sharma and Rao, (2012) explained the insecticide use in leafy vegetables of Jaipur region. They perform survey method to find out the use of insecticide by vegetable cultivator in Jaipur region.

The interaction of nanoparticles with forensic samples for their identification is explained by Rawtani et al., (2019). The article discussed latent fingerprints, explosive materials, pesticides, drugs, and their detection techniques, including colorimetry, fluorescence, and volumetry.

Malarkodi et al., (2017) offered a method for organophosphorus pesticide detection using dipstick immunoassay (DIA) which is straightforward, competitive, and sensitive method. Kitazine and other residual pesticides are found in fruits and vegetables, and they pose a serious threat to global consumer food safety. The DIA and ELISA procedures were used to examine the matrix impacts of several food samples (grapes, oranges, tomatoes, cucumbers, and tomatoes) in flow. Atomic Forced Microscopy was used to validate the creation of gold nanoparticles with different morphologies and sizes that were bioconjugated to Ig G, the detecting agent. The food sample's varying concentration of organophosphorus was revealed by the membrane strips taking on a purple hue. Among the quartet of fruit liquids examined, the grape juice exhibits the highest absorbance, signifying the active detection of the bioconjugate gold nanoparticle. The ELISA approach is also used to demonstrate that the Kitazine contained in the grape juice has satisfactory accuracy and acceptable reproducibility. The Kitazine residues in food samples can be quickly detected using these ELISAs as useful qualitative procedures whose results were assessed visually. The production of nanoparticles from green waste material was described by use of the different kinds of green waste materials for synthesis of different nanoparticles (Ali and Hassaan, 2017).

Kumar and Kumar, (2014) used an aqueous extract of *Camellia Sinensis* (green tea) leaves to perform green synthesis of zinc oxide nanoparticles (ZnO NPs). They employed XRD and UV-visible spectroscopy methods to characterise the freshly made nanoparticles.

DESCRIPTION OF TOPIC

By compiling the information after reviewing the literature available the first objective is planned to find the most prominently used pesticide on spinach from Jaipur by surveying farmers and by performing the physical and chemical laboratory methods for the collected spinach leaf samples. Next objective involves the use of green waste material for synthesis of nanoparticles and followed by characterization of newly synthesized nanoparticles by using various characterization technique like UV-Visible

spectroscopy, IR spectroscopy, Atomic Force Microscopy, Dynamic Light Scattering and Scanning Electron Microscopy (Abdali et al., 2024). Once desired nanoparticles were synthesized these nanoparticles will be transferred to the biodegradable plant-based cotton strip/banana strip which is neutral in nature. The objective is to develop a rapid detection device for pesticide detection on spinach.

Final objective is focused on to find out the interactive Mechanism of newly synthesized nanoparticles attached on plant based nanostrips with the pesticide as an *in-situ* detection device for spinach vegetables. to detect desired pesticide.

OBJECTIVE OF RESEARCH

Main objectives of the proposed research work are listed below:

- To survey and collect the data of Commonly/ prominently used pesticides on Spinach *(Spinacia oleracea* L.*)* by the farmers.
- To synthesize and screen nanoparticles from plant waste material as a safer and sustainable alternative for detection of pesticides.
- To do characterization of plant-based nanoparticles by using various tools and techniques.
- To study the interaction of newly synthesised plant-based nanoparticles with targeted pesticide.

RESEARCH METHODOLOGY

- **Sample Collection:** The Spinach leaf samples will be collected following survey of vegetable shops, Mandis, Departmental Stores of Jaipur region as well as agricultural sites of Jaipur area where farmers cultivate Spinach vegetables in their farms in and around different regions of Jaipur in different seasons. Physical and Chemical analysis will be carried out for the collected samples after handpicking and swabbing method (Singh et.al., 2020).
- The 2nd objective is focused on the synthesis of nanoparticles from the plant origin waste materials. Synthesis and screening of nanoparticles will be done through Bottom Up and Top-Down Method (Yusuf et al., 2022).
- The above synthesized and best reported nanoparticles will be characterised by Xray diffraction (XRD), Scanning Electron Microscopy (SEM), Atomic Forced Microscopy

(AFM), Fluorescence Spectroscopy, UV and IR Spectroscopy, DLS (Dynamic Light Scattering) (Abdali et.al., 2024).

- The mechanism of nanoparticles with the targeted pesticide will be done by colorimetric assay (Rawtani et al., 2019).

Colorimetric Assay: Chemical techniques known as colorimetric assays employ chemicals that change colour when they interact with an analyte. The target material and a detecting chemical combine to produce a colour. The compound's spectral absorbance at a particular wavelength is measured by a colorimeter. The chemical compound's concentration in the solution is ascertained by the colorimeter (Rawtani et al. 2019).

EXPECTEDOUTCOMEOF THE RESEARCH

The proposed study is focused to give the following expected outcomes

- Prominently pesticide used by farmer on spinach in Jaipur area will be identified and survey will help to find out the most common and most hazardous pesticide used in Jaipur region.
- Plant based green waste nanoparticle will be screened & synthesized which will be sustainable and will be useful for detection of pesticide in criminal investigation.
- Most prominently characterisation techniques will be identified for green waste-based nanoparticles.
- Mechanism of NPs and pesticide from forensic sample will be studied.

SIGNIFICANCE OF THE STUDY

- Nanoparticles play a crucial role in the detection of various types of pesticides from various exhibits.
- Nanoparticles provide improved sensitivity and selectivity in detection method.
- This study aims to investigate the potential of nanoparticles in detection of pesticides from the green leafy vegetables.
- Development of Nanoparticles based detection methods reduces the need & use of hazardous chemicals and helps in minimizing waste generation in the ecosystem.
- Use of nanoparticle-based detection method reduces the required sample size.

WORK PLAN

S. No.	Research Activity	6	12	18	24	30	36	*
1.	Course Work							
2.	Literature Survey							
3.	Methodology Steps							
4.	Quarterly Reports							
5.	Communication of Papers							
6.	Preparation of Final Thesis							
7.	Submission of Thesis							

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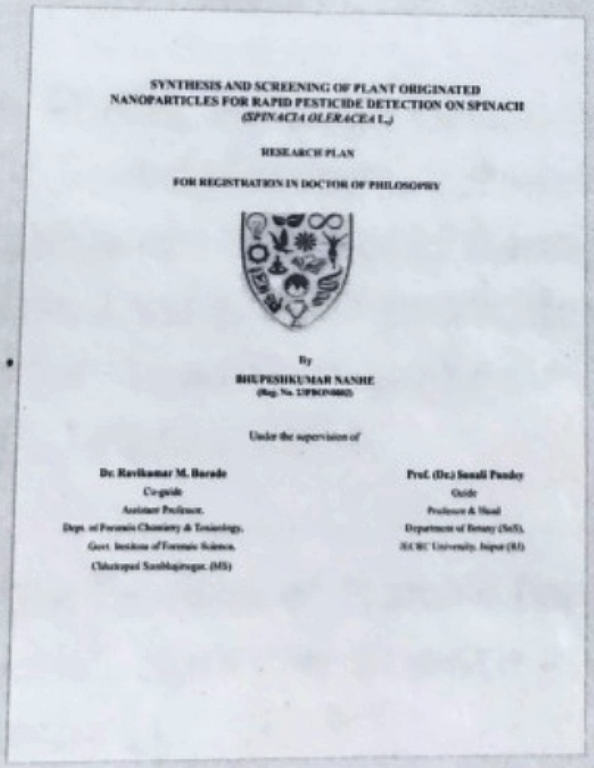

SYNTHESIS AND SCREENING OF PLANT ORIGINATED NANOPARTICLES FOR RAPID PESTICIDE DETECTION ON SPINACH (*SPINACIA OLERACEA* L.)

RESEARCH PLAN

FOR REGISTRATION IN DOCTOR OF PHILOSOPHY

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